REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

Claims 1-17 have been cancelled without prejudice and new claims 18-45 added in lieu thereof. Claims 18 and 35 are drawn to methods of treatment and pharmaceutical compositions based on the carboxylic acid esters of Erythromycin B and Erhthromycin B enol ether (see comments that follow). Claims 36-39 are drawn to novel Erythromycin B enol ether esters. Claims 40 and 41 are drawn to novel 2'-esters of Erythromycin B with a dicarboxylic acid. Claims 42-45 are drawn to methods of treatment involving the use of Erythromycin B. The claims as presented are fully supported by an enabling disclosure.

An Abstract has been provided, as required.

Prior to addressing the specific rejections, the following comments are offered by way of providing background for the present invention.

The present invention is based around certain findings relating to Erythromycin B which is a macrolide antibiotic having the formula shown as compound (4) in Fig. 1 of the present application. However before considering these effects, attention is directed to Erythromycin A, for which the formula is shown as (1) in Fig. 1 of the present application, and comments that follow.

Erythromycin A has been used clinically as an antibacterial agent which is administered orally. However despite its long success, Erythromycin A exhibits a number of disadvantages. Two such disadvantages may be cited for present purposes.

One such disadvantage is its vile taste which results in poor patient compliance especially in children. This disadvantage of Erythromycin A has been addressed by the use of 2' esters with a carboxylic acid, e.g. the Erythromycin A ethyl succinate ester (EAES) shown as compound (5) in Fig. 2 of the present application. These esters mask the taste of Erythromycin A but suspensions of these esters (e.g. for administration as a paediatric formulation) are subject to degradation during storage (particularly if the formulation is not kept in a fridge) resulting in partial conversion of the ester in the formulation to Erythromycin A with its attendant taste problems. The other disadvantage is that Erythromycin A is acid sensitive and it (as well as its ester derivatives) is degraded in the acid conditions of the stomach. The degradation products do not have antibacterial activity but they do cause gastric disturbance and are metabolized in the liver (see paragraph bridging pages 1 and 2 of the present specification). This degradation in the acid conditions of the stomach also applies to the ester derivatives (e.g. the EAES referred to above) since it is the macrolide ring system itself which is degraded by the acid. The degradation of the ester in the stomach is also a problem because the ester needs to pass into the intestine and be absorbed for conversion by a base-catalysed mechanism to Erythromycin A which is the active compound. Thus if the ester is degraded in the stomach it cannot pass into the intestine for conversion into the active form.

Due to the above, relatively large doses (e.g. up to 2g per day) are required for efficacy because (as will be appreciated from the above comments) a high proportion of the administered drug is degraded in the acid conditions of the stomach.

Turning now to Erythromycin B, prior to the priority date of the present application, it was known that Erythromycin B had antibacterial properties (and in fact there is *in vitro* data to this effect in some of the citations raised by the Examiner) but it was not used in clinical practice. Applicants found that Erythromycin B has considerably enhanced acid stability as compared to Erythromycin A so that Erythromycin B (and its derivatives – discussed below) would be a very suitable replacement for Erythromycin A as an orally administrable antibiotic. The acid stability of Erythromycin B is adequately demonstrated by the degradation studies reported in the text of the present application. This enhanced acid stability of Erythromycin B (which "carries through" to its derivatives – discussed below – because it is a property of the macrolide ring system) means that a much higher proportion of the drug remains "intact" in the stomach. By "intact", it is meant that the integrity of the Erythromycin B ring system is maintained although there may be some "loss" of substituents in the case of certain derivatives – see below). This acid stability (in the stomach) of the Erythromycin B ring system gives rise to a number of features which are discussed below.

With regard to oral administration of Erythromycin B itself (which also has an unpleasant taste), this will remain substantially intact (as an antibiotic) in the stomach before passing into the intestine. The fact that the Erythromycin B remains intact in the

stomach means that it can be used for the treatment of "stomach resident" bacteria, an important example of which is *Helicobacter pylori*. Additionally the amount of Erythromycin B that must be administered as a therapeutically effective dose (either for treatment of a stomach resident or other bacteria) is much less than is required for Erythromycin A. Thus a daily dose might be a maximum of 500mg per day as compared to 2 grams per day as generally employed for Erythromycin A (see second complete paragraph on page 3 of the present specification. This has advantages with regard to patient compliance. Furthermore since the Erythromycin B is stable in the stomach there are no substantial amounts of degradation products giving rise to adverse reactions.

While it is possible to identify the advantages (summarized in the previous paragraph) with regard to administration of Erythromycin B *per se*, the importance of the present invention does reside in "taste masked" derivatives of Erythromycin B which are particularly important for pediatric formulations.

One set of such derivatives are the 2' – carboxylic acid esters of Erythromycin B (see 5th and 6th paragraphs on page 4 of the present specification). A particular example of such an ester (although not specifically mentioned in the present specification) would be Erythromycin B ethyl succinate which is the "Erythromycin B equivalent" of Erythromycin A ethyl succinate shown in Fig. 2 of the present specification. The following half-life data demonstrate the enhanced stability of Erythromycin B ethyl succinate compared to Erythromycin A ethyl succinate over a range of pH conditions that are likely to be encountered in the stomach.

Ery B ethyl succinate at pH 2	approx 70 mins
Ery A ethyl succinate pH 2	undetectable (ie < 5 mins)
Ery B ethyl succinate pH 2.5	> 3 hours
Ery A ethyl succinate pH 2.5	undetectable
Ery B ethyl succinate pH 3	> 7 hours
Ery A ethyl succinate pH 3	< 10 mins
Ery A ethyl succinate pH 3.5	58 mins
Ery A ethyl succinate pH 4	2 hours 50 mins

All half lifes are for degradation to inactive products.

No result is presented for Ery B B ethyl succinate at pH values of 3.5 and 4 since it will be "rock stable".

The above data clearly demonstrate the stability of 2' carboxylic esters of Erythromycin B in the stomach. The ester itself would then pass to the intestine for absorption and conversion to Erythromycin B (by base-catalysed cleavage of the ester group) to generate free Erythromycin B as the active antibiotic.

For the reasons outlined in the previous paragraph, the 2' esters of Erythromycin B are therapeutically useful antibiotics since they do not generate substantial amounts of degradation products (gut motility agents) as would Erythromycin A. There is however a disadvantage with the 2' – carboxylic esters of Erythromycin B in that when formulated as an orally administrable suspension (particularly for pediatric administration) there is some degradation to Erythromycin B during storage. Thus there is some loss of the "taste masking" during storage. This disadvantage is overcome by the further derivatives of Erythromycin B discussed in following paragraph.

Additional Erythromycin B derivatives contemplated by the present invention are the 2' carboxylic esters of Erythromycin B enol ether. A particular example of such an

ester (although not specifically mentioned in the present specification) is Erythromycin B enol ether 2'-ethyl succinate for which the structure is shown on the attached sheet. In the acid conditions of the stomach, the Erythromycin B enol ether 2'-ethyl succinate is rapidly converted to Erythromycin B ethyl succinate so that (in the stomach) the same advantages apply as are discussed above in relation to Erythromycin B ethyl succinate. The additional advantage of Erythromycin B enol ether succinate lies in its stability in orally adminstrable suspensions. To establish the stability of Erythromycin B ethyl ether 2' ethyl succinate in formulation conditions, a comparative degradation study of Erythromycin B ethyl succinate (EBES), Erythromycin A ethyl succinate (EAES) and Erythromycin B enol ether 2' ethyl succinate (EBEES) mimicking the conditions of formulation for pediatrics and storage was developed. In order to determine the effect of pH and solubility on the extent of hydrolysis, the compounds were formulated as suspensions (25 mg ml^{-1}) and were stored in a refrigerator at 4°C for 21 days. Subsequently the samples were extracted with chloroform and one dimensional ^{1}H spectra where acquired. Signals at 3.20 (H-2' in the free drug) and 4.75 (H-2' in the ester) were used to determine the extent of hydrolysis. The results are shown in the following table.

Drug	Extent of hydrolysis to erythromycin A or erythromycin B (%)	
	pH 6.0	pH 8.0
EBES	68.2	23.1
EBEEES	0.0	0.0
EAES	26.2	30.1

Thus the storage stability of the 2'-ester of Erythromycin B enol ether is clearly demonstrated.

Claims 14-18 stand rejected under 35 USC 112, first paragraph, as allegedly being non-enabled. Withdrawal of the rejection is submitted to be in order in view of the above-noted claim revisions and comments that follow.

The rejection of claims 14-18 (to the extent that they previously broadly covered "microbial" (again e.g. viral) infections) has been overcome by limiting the claims to bacterial infections.

The Examiner also contends that the specification does not provide enablement for treatment of TB, Syphilis, Helicobacter pylori or Chlamydia. However, the Examiner offers no evidence to support the assertion. As the Examiner is aware, an applicant enjoys benefit of the presumption that the invention can be practiced as claimed. The burden is on the Examiner to provide evidence to the contrary.

In view of the above, reconsideration is requested.

Claims 1-18 stand rejected under 35 USC 112, second paragraph. Withdrawal of the rejection is submitted to be in order in view of the above-noted claim revisions. Reconsideration is requested.

Claims 14 and 17 stand rejected under 35 USC 101. Withdrawal of the rejection is in order in view of the above-noted claim revisions. Reconsideration is requested.

Claims 1-18 stand rejected under 35 USC 102(b) as allegedly being anticipated by, or, in the alternative, under 35 USC 103 as allegedly being obvious over, EP-A-0 553 353, Bojarska-Dahlig (Quant. Struct.-Act. Anal), Bojarska-Bahlig et al (Pol. J. Pharm.), Ono et al, Cane et al, Kibwage et al or Martin et al. The rejection is traversed.

Martin et al discloses, in Table (III), the antibacterial activity of Erythromycin B (compound B) and its 2'-acetyl ester (compound 2B). Table (VII) discloses *in vitro* data for Erythromycin B but not the 2'-acetyl ester. With regard to the 2'-acetyl ester, the data shown in Table (III) is, as indicated, *in vitro* data and demonstrates that the ester is less active than the parent Erythromycin B. In this respect, compare the "Log potency" values for compounds B and 2B as listed in Table (III) for which it will be seen that compound 2B is considerably less active than Erythromycin B itself. There is no disclosure or suggestion in this document of the stability under acid conditions (as encountered in the stomach) of Erythromycin B. For this reason, there is no pointer to the advantages that are obtained by use of the 2' esters of Erythromycin B so that their use as a therapeutic agent would not have been rendered obvious by the disclosure of this citation, particularly, also since the *in vitro* data shows the ester to be less active than

Erythromycin B. Furthermore, the particular subject matter of claims 42-45 (relating to use of Erythromycin B *per se*) would not have been rendered obvious because (as indicated) the subject matter of these claims relies on an appreciation of the stability under acidic conditions of Erythromycin B and there is no pointer to this feature in the citation. There is no disclosure or suggestion in the citation of the esters of the Erythromycin B enol ether.

Ono et al discloses antibacterial activity of Erythromycin B (see second compound in Table 2) and also some derivates thereof. There is no reference to the 2'-esters of Erythromycin B or Erythromycin B enol ether. This citation is not relevant to claims 18-41. There is no reference to the stability under acid conditions of Erythromycin B so the comments offered above in relation to claims 42-45 apply.

Bojarska-Dahlig et al (Pol. J. Pharm.) discloses biological potency of Erythromycin B and some derivatives thereof (see Table I). There is no reference to 2'-esters. Overall, therefore, similar comments apply to this citation as for those above.

Kibwage et al discloses antibacterial activity of Erythromycin B and Erythromycin B enol ether against a range of gram-positive and gram-negative micro organisms. There is no reference to the stability under acidic conditions of Erythromycin B nor any reference to 2'-esters. Thus similar comments as previously apply.

Cane et al relates to macrolide biosynthesis. There is a reference in Table I to the 2'-benzoate of Erythromycin B but not as a pharmaceutical but rather for the purposes of investigating the stereochemical course of the chain-elongation steps in the biosynthesis

reaction (see the Abstract of the citation). There is no reference to the 2'-esters of Erythromycin B or Erythromycin B enol ether with a dicarboxylic acid. The reference in the left hand column on page 4961 to the incorporation of labelled succinate into erythromycin is as a biosynthetic precursor of the erythromycin. There is no reference to the stability under acid conditions of Erythromycin B. Consequently similar comments as previously apply.

EP-A-0 553 353 relates to erythromycin derivatives having a carbonate group at the 2'-position. Example 10 relates to the production of the carbonate of Erythromycin B and, in fact, Erythromycin B is referred to in claim 2. There is no reference to the 2'-esters of Erythromycin B or Erythromycin B Enol Ether with a carboxylic acid. There is no reference to the stability under acid conditions of Erythromycin B. Therefore similar comments as previously apply.

Bojarska-Dahlig (Quant. Struct. – Act. Anal.) discloses biological potency of Erythromycin B (see Table I). There is no reference to the 2'-esters of Erythromycin B or Erythromycin B enol ether. There is no reference to the stability under acidic conditions of Erythromycin B. Consequently the same comments as previously apply.

Reconsideration is requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

BARBER et al
Appl. No. 10/018,467
September 8, 2003

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: Mary J. Wilson
Mary J. Wilson
Reg. No. 32,955

MJW:tat
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

The key intermediate in our synthesis, (3) was readily prepared using the published protocol.[4] Treatment of (3) with the appropriate acid chloride,[5] yielded the 2'-ester, erythromycin B 2'-enol ether ethyl succinate (4) as shown in Figure 3.

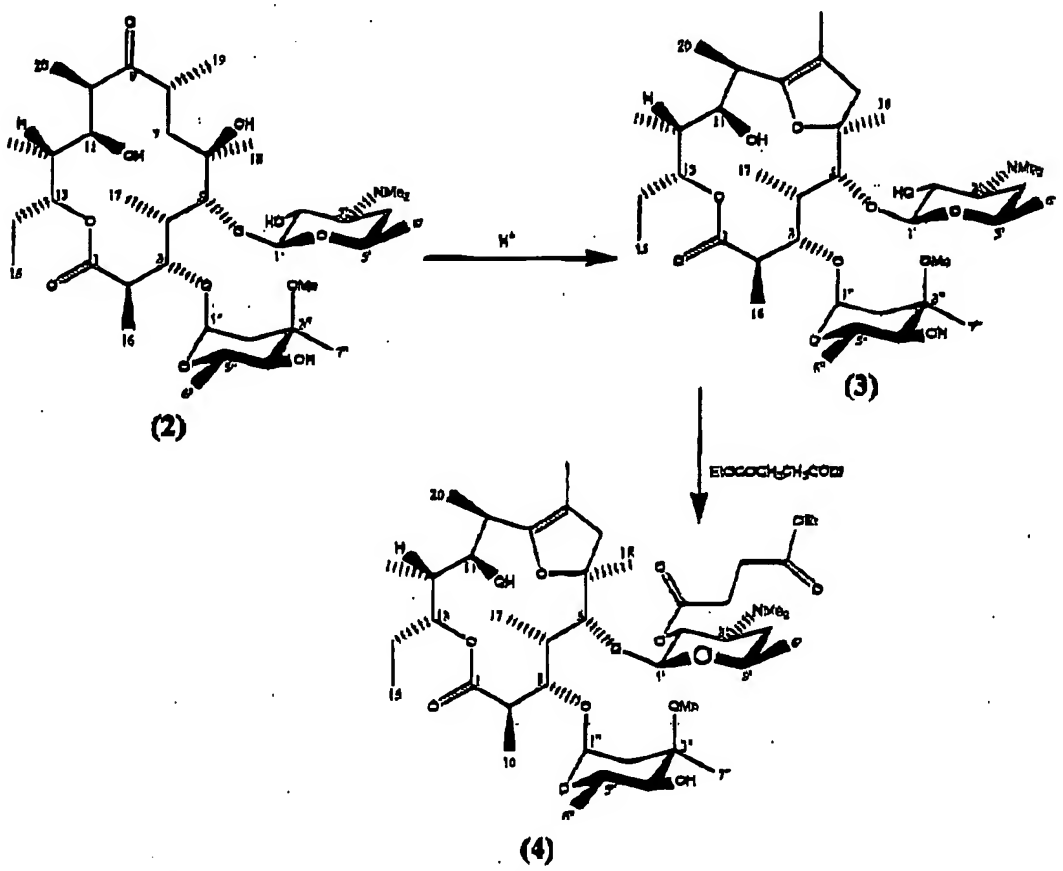

Figure 3: Synthesis of erythromycin B enol ether 2'-ethyl succinate

Solubility Studies

Erythromycin esters are absorbed intact in the intestine[6] but require base catalysed hydrolytic conversion to free erythromycin in order to impart antibacterial activity. This process is dissolution rate limited and is equally applicable to the hydrolysis occurring in the medicine bottle. Relative solubities of (4) at pH 2.0, 6.0 and 7.0 was determined using NMR and was compared with those of (2), EAES and EBES.

Drug	Solubility (mM)		
	pH 2.0	pH 6.0	pH 7.0
Erythromycin B	>30	25.5	19.9
EBES	>30	5.33	1.20
EAES	6.6	1.94	0.83
EBEEES (4)	>30	0.3	A